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THE VELIGER

Early Development of *Crassostrea iredalei* (Faustino, 1932) (Bivalvia: Ostreidae), with Notes on the Structure of the Larval Hinge[1]

by

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Abstract. Larvae of the oyster *Crassostrea iredalei* were reared in the laboratory from eggs through settlement. The oysters were induced to spawn by increasing the temperature by 5–10°C and sometimes by adding stripped oyster sperm to the spawning dishes. Eggs averaged 48 μm in diameter.

The straight-hinge veligers appeared 22 to 26 h after fertilization. The larval shell length increased from 64 to 84 μm in the straight-hinge stage, from 85 to 275 μm in the umbo stage, and from 210 to 275 μm in the pediveliger stage. Eye-spotted pediveligers were observed mostly at lengths greater than 225 μm. The hinge line did not increase much with larval growth. Although length was initially greater than height, the increase in height was much faster due to the development of the umbo. Height was greater than length in more advanced larvae. Valve growth was asymmetrical and unequal, with the left valve generally larger. Settlement and metamorphosis occurred 20 days from fertilization at lengths of 270 μm and greater, when the oyster larvae were reared at 26.5 to 30°C and salinities of 30 to 32 ppt.

The larval hinge structure consisted of minute dentition on the central portion of the provinculum and large rectangular teeth on both ends. These teeth became obscured in advanced larvae due to the skewed development of the umbo.

Data derived from the laboratory culture of larvae of *Crassostrea iredalei* may be used in spatfall forecasts for the collection of larvae from the wild and as baseline information for the hatchery culture of oyster larvae.

INTRODUCTION

Crassostrea iredalei (Faustino, 1932) is a large, non-incubatory oyster common in most estuarine and plankton-rich marine waters of the Philippines (Carreon, 1969). It is a fast-growing species and is the most economically important mollusk in the country.

The biology and ecology of *Crassostrea iredalei,* as well as its farming aspect, have been well studied (Villaluz, 1938; Blanco *et al.,* 1951; Blanco & Montalban, 1955; Escritor, 1962; Carreon, 1973) and the work of Carreon (1969) has clarified its position in the family Ostreidae. However, the larval biology of *C. iredalei* has hitherto been unreported, leaving a gap in the knowledge of its life history.

The recruitment period of new populations of oysters for cultivation may be forecasted by monitoring (1) the start and duration of the spatfall period, (2) the intensity of the spatfall, and (3) the occurrence and abundance of oyster larvae in the plankton (see Quayle, 1969:65). Techniques for a scientific and effective spatfall forecast for oysters in the Philippines have not yet been well established. Thus, local oystermen make spatfall predictions based only on previous experience and traditional practices. This probably is a primary reason for the slow development of the Philippine oyster farming industry.

This study describes the development of laboratory-reared larvae of *Crassostrea iredalei* through metamorphosis. The results should enable positive identification of the larvae in the plankton and the determination of its stages of development. Data derived from this study should help in establishing a good spatfall forecasting program, which is an essential aspect of the culture and farming of this valuable Philippine food resource.

[1] SEAFDEC Aquaculture Department Contribution No. 158.

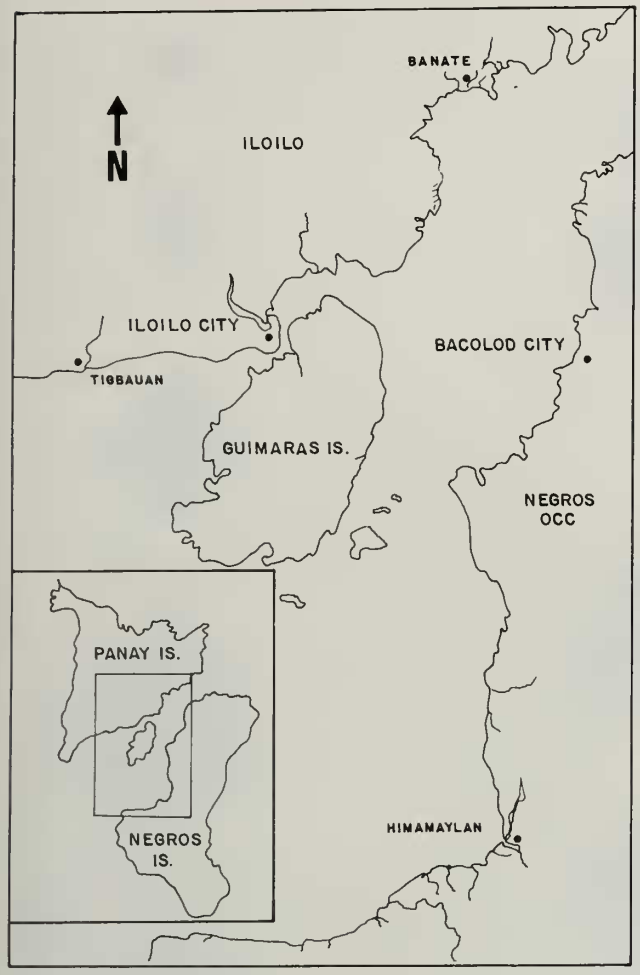


Figure 1

Map of Negros and Panay Islands. Collection areas for broodstock of the oyster *Crassostrea iredalei* (Banate, Iloilo, and Himamaylan, Negros Occidental) and the SEAFDEC Tigbauan Research Station.

MATERIALS AND METHODS

Sexually mature adult oysters, *Crassostrea iredalei* (50–80 mm long), were collected from SEAFDEC experimental farms in Himamaylan, Negros Occidental, and Banate, Iloilo. They were then transported to the SEAFDEC Tigbauan Research Station, Tigbauan, Iloilo (Figure 1). Oysters from the Himamaylan area were gathered from growing cultches suspended from floating rafts while those from the Banate site were gathered from farms that use the lattice method of oyster culture. In the lattice method of culture, the growing ropes for oysters were hung horizontally, radiating from a central point about 0.5–1 m from the bottom.

The newly gathered oysters were immediately cleaned of dirt and fouling organisms and, when possible, separated from each other. The oysters were "cold-conditioned" in tanks by maintaining the seawater temperature at 21 to 23°C with the use of a thermostatically controlled immersion water cooler for at least three days before they were induced to spawn. The oysters were fed mixed plankton prepared by the SEAFDEC Phycology Laboratory.

Table 1

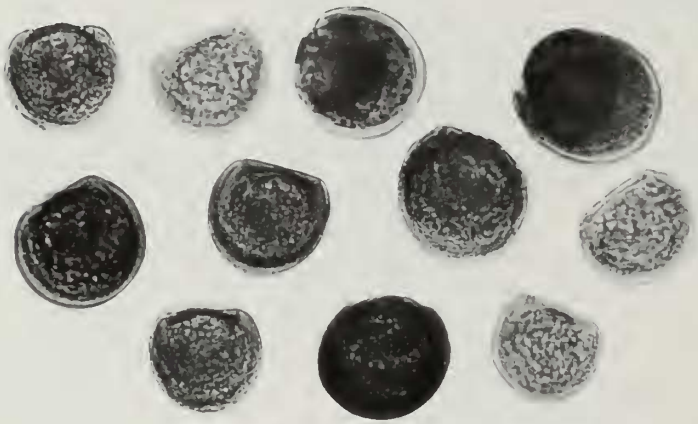
Summary of embryonic development periods of *Crassostrea iredalei* (temperature, 27–28°C; salinity, 32 ppt). Stages as described by BAYNE (1965).

Stage	Description	Time
0	fertilization	0
1	first division	45–50 min
2	ciliated blastula	5.5 h
3	early trochophores	8 h
4	veliger	15 h
5	transitional stage	18–20 h
6	straight-hinge stage	22–26 h

Cold-conditioned oysters were induced to spawn by quickly increasing the temperature by 5 to 10°C and sometimes by adding stripped oyster sperm to the spawning dishes. After a latency period of 2 to 6 h, some males emitted gametes which often stimulated the oysters to spawn en masse. The fertilized eggs were collected and washed of excess sperm with freshly filtered seawater.

The basic techniques used in rearing the oyster larvae were as described by LOOSANOFF & DAVIS (1963), CHANLEY (1975), and CULLINEY *et al.* (1975). Larval cultures were kept under shelter but at ambient temperature and salinity (26.5–30°C and 30–32 ppt, respectively). Fiberglas and polyethylene pails (100-L capacity) and glass beakers (1–5-L capacity) were used as culture vessels. Aeration was provided only when the larvae were reared in the large culture tanks. The fertilized eggs were initially stocked in the culture vessels at a density of 30 eggs/ml of culture water and were left undisturbed for the first 24 h. After this period and every second day thereafter, the culture water was changed and larval food was added. The larval food used was *Isochrysis galbana,* which has been shown to be one of the best foods for bivalve larvae (DAVIS & GUILLARD, 1958). Food was provided at a density of 3×10^4 cells/ml of culture water in the first week of rearing, 5×10^4 cells/ml in the second week, and 8×10^4 cells/ml in the third week.

During each water change, healthy larvae swimming in the water column were collected by siphoning the culture water through a series of nylon sieves. The mesh sizes of the sieves were determined by the range of sizes of the larvae in the culture. Because debris and the dead and moribund larvae mostly settled at the bottom of the culture vessels, the last 5-cm layer of water was not siphoned off. However, to rescue any healthy larvae that might be mixed



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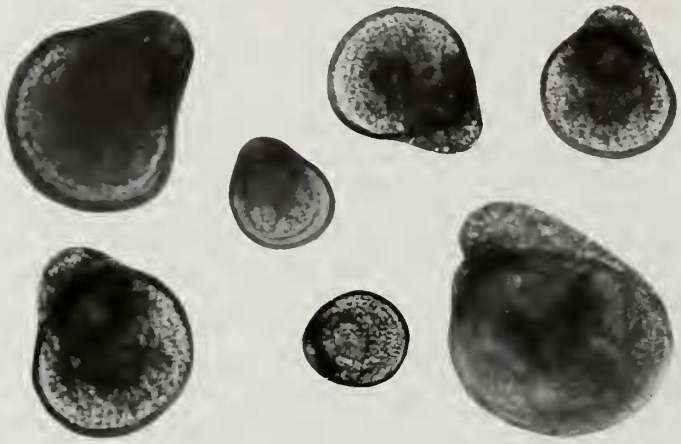

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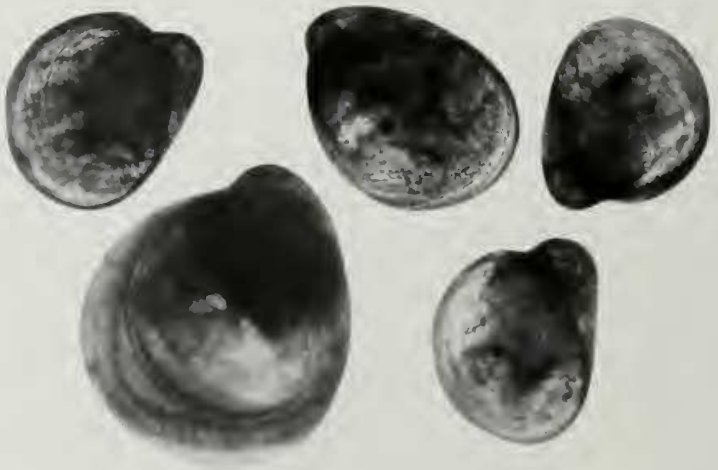

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Table 2

Main features of the larval development of *Crassostrea iredalei* (temperature, 26.5–30°C; salinity, 30–32 ppt).

Stage	Age	Mean size (μm)	Range (μm)	SE (μm)	n	Remarks
Straight-hinge veliger	22 h–4 days	L = 74	64–84	0.528	110	D-shaped; shell asymmetrical but equivalved
		H = 67	56–80	0.708	110	
		D = 48	41–61	0.948	41	
		Hinge-line length = 50				
Umbonate veliger	5–22 days		L = 85–275		419	rounded umbo at lengths 85–90 μm; knob-like umbo at lengths 91–150 μm; skewed umbo at lengths greater than 150 μm
			H = 81–305		422	
			D = 62–220		178	
Pediveliger	16–22 days		L = 210–275		18	alternately creeping and swimming vigorously
			H = 240–305		15	
			D = 171–220		6	
Eye-spotted pediveliger	18–22 days	L > 217				larvae nearing metamorphosis; eye-spot diameter, range 8–15 μm
		H > 242				
Spat	20 days	L > 274				larvae attached on cultches
		H > 328				

with the dead and moribund larvae, the "bottoms" were cultured in a separate vessel (CULLINEY *et al.*, 1975). Once the larvae reached the pediveliger stage, cultches made from adult oyster, scallop, and window-pane oyster shells were hung in the water or placed at the bottom of the culture vessels. Some larvae were transferred to petri dishes or finger bowls to facilitate observation of their settlement.

Larval samples were collected at each water change, fixed in "sucrose-formalin preservative" (CULLINEY *et al.*, 1975), and measured, photomicrographed, and studied for their shell characters. All dimensions of the larvae were measured using a calibrated filar micrometer. The descriptive terminologies used were as defined and illustrated by CHANLEY & ANDREWS (1971).

To facilitate observation of the hinge structure, the valves of the preserved larvae were separated by immersing the larvae in a solution of 10% sodium hypochlorite for a few minutes and repeatedly washing them with distilled water (REES, 1950) after which they were mounted on a depression slide.

RESULTS AND DISCUSSION

Induced Spawning

The conditioning technique for *Crassostrea iredalei* employed in this study was done to prepare the breeding stock for induced spawning and not necessarily to stimulate gametogenesis, as was practiced by Loosanoff and colleagues (LOOSANOFF & DAVIS, 1963). The cold water (21–23°C) actually prevented spontaneous spawning by the oysters after being transported in the dry state from the collection areas. Most of the mature oysters with ripe gonads readily spawned in the laboratory after they were stimulated thermally or with the addition of sperm.

The addition of stripped sperm into the spawning dishes almost always elicited a positive spawning response, beginning mostly with the males and then the females. The relative ease in which spawning was induced may also indicate the actual gametogenic stage of the gonads since reproduction in *C. iredalei* is continuous (SEAFDEC, unpublished data). This sensitivity to stimulation augurs well for oyster propagation in that it facilitates the mass spawning of the population.

Oysters were fed a daily ration of mixed phytoplankton in order to minimize deleterious effects on the produced larvae should the breeding stocks be starved. HELM *et al.* (1973) have shown experimentally that in *Ostrea edulis*, larval vigor is definitely enhanced when the breeding stock is not nutritionally stressed.

Embryonic Development

Unfertilized eggs of *Crassostrea iredalei* were pear-shaped and white in color. The eggs soon became spherical upon

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Figure 2

Composite photomicrographs of *Crassostrea iredalei* larvae. Length × height measurements are given in μm under each individual larva at right. Larvae are arranged with the anterior end at the right. A. Straight-hinge and early umbonate larvae, 1–5 days old. B. Group of umbonate larvae, 5–20 days old. C. Eye-spotted pediveligers, 20 days old. Note newly settled larva showing distinct dissoconch growth in bottom left photograph.

Table 3

Comparison of the major features of *Crassostrea iredalei*, *C. gigas*, and *C. virginica*.

Stage or distinctive feature	*C. iredalei*	*C. gigas*[1]	*C. virginica*[2]
Straight-hinge veliger	64–84 μm	70–90 μm	68–90 μm
Conspicuous umbo			
Rounded	91–95 μm	90 μm	80–100 μm
Knobby	100–110 μm	125 μm	85–105 μm
Skewed	150 μm	150–200 μm	125 μm
Length equals height	85–90 μm	—	90–100 μm
Attached spat	>274 μm	300 μm	310–350 μm

[1] Data from LOOSANOFF *et al.* (1966) and CHANLEY & DINAMANI (1980).

[2] Data from LOOSANOFF *et al.* (1966) and CHANLEY & ANDREWS (1971).

fertilization and ranged in diameter from 47 to 50 μm (average, 48 μm). No measurements were made of the spermatozoa.

The periods of embryonic development up to the straight-hinge stage are presented in Table 1, with embryonic stages as described by BAYNE (1965). The approximate time of occurrence for each stage is given in the number of minutes and hours from fertilization, with fertilization time being time zero.

The formation of the first polar lobe, 45 min after fertilization marked the start of cleavage. Blastulation was observed in embryos older than 2 h and the ciliated blastula stage was reached in 5–6 h. At this stage the embryos started to roll and rotate. Early trochophores were observed 8 h after fertilization and were fully developed after an additional 7–8 h. The trochophores later developed into straight-hinge veligers in 22–26 h from fertilization.

Larval Dimensions and Shapes

The main features of larval development are summarized in Table 2. The changes in the shape and size of the larvae from the straight-hinge veliger to the newly metamorphosed spat are illustrated in Figure 2.

The smallest straight-hinge veliger observed was 64 μm long and 57 μm high, although one-day-old veligers averaged 69 × 60 μm. The hinge-line length averaged 50 μm. The larvae were in the straight-hinge stage until the length equaled the height.

The height grew faster than the length, which in turn grew faster than the depth. Initially, the height was 5–10 μm less than the length, but these became equal when the larvae were 85–90 μm long. Eventually, the height exceeded the length by as much as 30 μm in older larvae. The depth was 10–15 μm less than the length in the straight-hinge larvae and 90–100 μm less in the pediveliger stage.

The straight-hinge larvae were typically D-shaped, asymmetrical, and equivalved. The anterior margin of the shell was more pointed than the posterior margin. The shoulders dropped sharply in the anterior portion but more gradually in the posterior portion. The ventral margin of the shell was well rounded toward the posterior portion.

The larvae at length 85–90 μm appeared to be circular when lying on one valve due to the development of a slightly rounded umbo. The umbo of the veligers at this point developed very rapidly. In larvae 91–96 μm long, the umbos were already well rounded or slightly knoblike. The umbos became well developed knobs on the dorsal portion in larvae 100–110 μm long and eventually became skewed when the larvae were 150 μm long. The skewed umbo was characterized by CHANLEY & ANDREWS (1971) to be a variation of the "knobby" type and is found only in the genus *Crassostrea*. This larval shell characteristic of the *Crassostrea* bears much significance in the identification of the larvae of the various oyster species in the plankton.

The valves of the shell grew asymmetrically due to the highly unequal growth of the umbo, with the left valve much higher than the right by as much as 25 μm in the late stages. The hinge line did not lengthen much with additional growth of the larvae and could be discerned only until larvae were 95–100 μm long. As the umbo developed, the hinge line became more obscured.

In the pediveliger stage, the anterior portion of the shell was longer, more pointed, and was much lower than the posterior portion. The drop of the anterior margin from the shoulder was gradual and long, whereas that of the posterior margin was short, abrupt, and curved. The larvae were now alternately creeping and swimming vigorously.

The relationships of the larval dimensions and the derived regression lines are shown on the scatter diagrams in Figure 3. Each regression line describes two distinct linear relationships between the larval dimensions, with a breakpoint occurring at about the time of the rapid development of the umbo. The results here show much similarity to the growth of *Crassostrea virginica* and *C. gigas* as reported by LOOSANOFF *et al.* (1966) (see Table 3). The growth data for all three species indicate the same distinct linear relationships of the larval dimensions with a break point appearing at lengths between 115 and 125 μm.

It has been reported that *Crassostrea gigas* was imported from Japan in 1963 and experimentally farmed in southern Philippines (D. K. Villaluz, personal communication). Thus, some difficulty may arise in the identification and isolation of *C. iredalei* and *C. gigas* larvae should both these species occur together in the plankton, as experienced by LOOSANOFF *et al.* (1966) when *C. virginica* and *C. gigas* occurred together. It is also possible that these

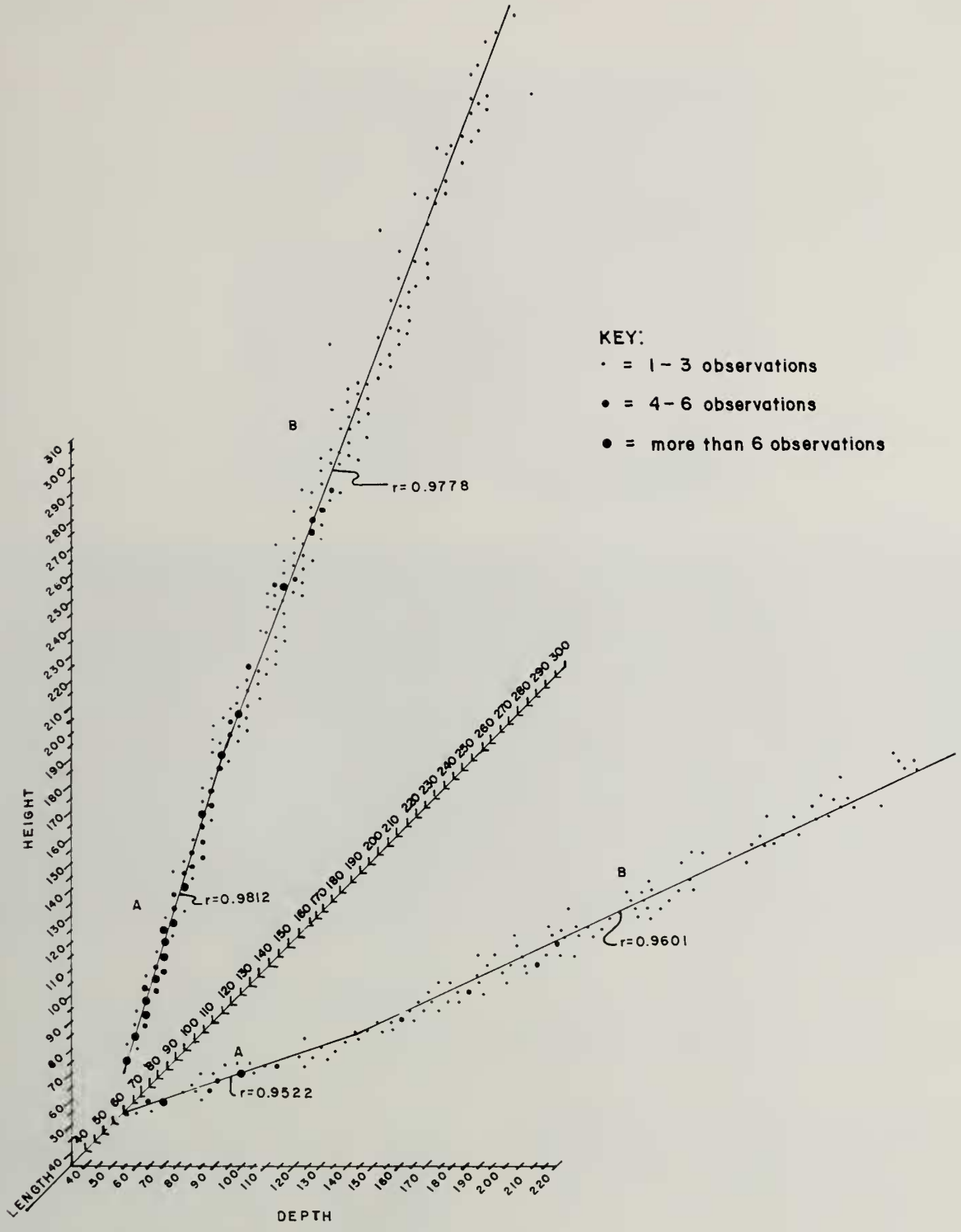

Figure 3

Dimensions of larval *Crassostrea iredalei*. Dots represent observed length-height or length-depth measurements. Height and depth coordinates run parallel to length axis. Regression lines show length *vs.* height and length *vs.* depth relationships. Regression equations are, for length-height: (A) $H = 1.5726 \times L - 49.1153$, $r = 0.9812$, (B) $H = 1.1319 \times L + 1.4758$, $r = 0.9778$; and for length-depth: (A) $L = 0.7352 \times D + 38.6897$, $r = 0.9522$, (B) $L = 1.3039 \times D - 17.0189$, $r = 0.9601$. The three-dimensional graph is adapted from CHANLEY & VAN ENGLE (1969).

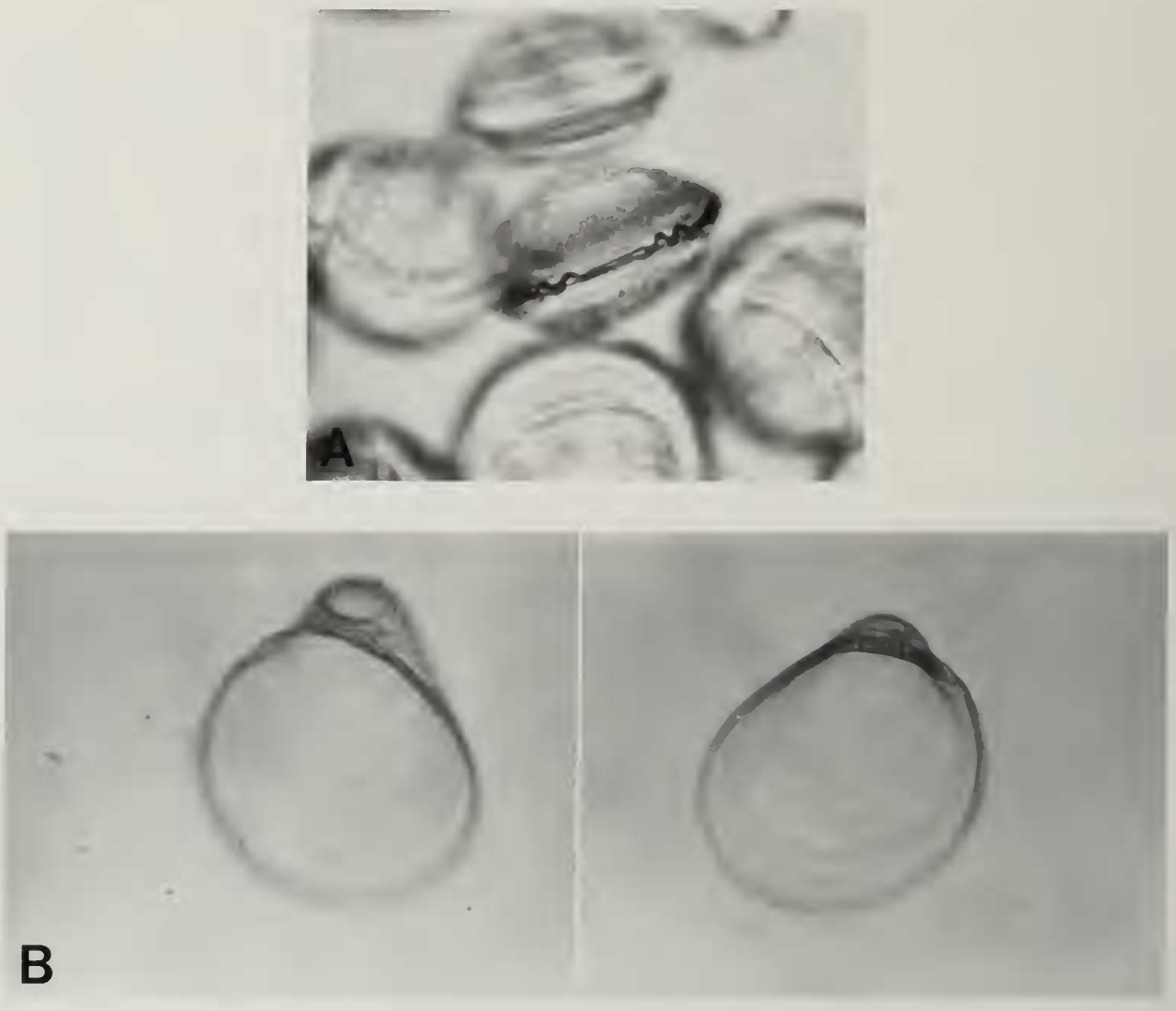

Figure 4

Larval hinge structure of *Crassostrea iredalei*. A. Dorsal view of straight-hinge larva, 89 × 83 μm, showing provinculum and hinge structure. B. Inside view of hinge structure of umbonate larval shell. Left valve (left) is 215 × 249 μm, with the anterior portion on the right. Right valve (right), 214 × 236 μm, anterior portion on the left.

two species have hybridized. Further studies on the biology of larvae caught from the wild, especially from the area of the southern Philippines, must be done.

Larval Hinge Structure

In straight-hinge larvae of *Crassostrea iredalei*, the hinge structure consisted of two to three large rectangular teeth on both ends of the provinculum and minute dentition on the central portion (Figure 4a). The left and right valves have very similar hinge structures, with the teeth perpendicular to the hinge line.

Due to the pronounced asymmetrical growth of the umbos of the left and right valves in umbonate larvae, the hinge structure of both valves changed progressively as the larvae matured. The slight coiling and skew of the umbo in the later stages had corresponding effects on the hinge structure.

The anterior portion of the larvae became longer, with the margin sloping more toward the ventral portion. As a result, the anterior group of hinge teeth angled inward and the posterior group of hinge teeth became obscured; in more advanced larvae, the most posterior tooth was much reduced. Likewise, the posterior margin of the valves became wider due to the coiling of the umbo (Figure 3b).

In late pediveligers, the posterior teeth of the left valve

were much reduced. The hinge line consequently slanted more toward the anterior, and the modified posterior margin became much wider.

The morphology of the larval shell, especially the hinge structure, was illustrated by DINAMANI (1976) as an important character in the systematic differentiation of the family Ostreidae at the subgeneric level. He extended the descriptions for the ostreid genera *Saccostrea* and *Crassostrea* provided by STENZEL (1971) to include distinctive features of the prodissoconchs. According to DINAMANI (1976), the prodissoconchs of the genus *Saccostrea* have "orthogyrate umbones and symmetrical teeth" whereas in the genus *Crassostrea* the prodissoconchs have "inequilateral growth, with posterior teeth modified and with the umbones tending to be opisthogyrate."

The prodissoconchs of *Crassostrea iredalei* definitely display the distinctive characters of the genus *Crassostrea,* and represent an intermediate position between *C. virginica* and *C. angulata-C. gigas* as can be gleaned from the illustrations of DINAMANI (1976).

The data derived from the laboratory culture of *Crassostrea iredalei* larvae may be used as baseline information for the mass cultivation of this bivalve in a hatchery grow-out system. However, a more important application of this study would be the use of the description of the larvae and knowledge of its biology for monitoring the start and duration of the spatfall period, its intensity, and the abundance of larvae in the plankton. These activities are necessary for the collection of oyster seeds from the wild for purposes of cultivation.

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